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**Published***With international search report.**Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*(54) Title: RAPID SYNTHESIS AND USE OF <sup>18</sup>F-FLUOROMISONIDAZOLE AND ANALOGS

## (57) Abstract

The present invention involves a rapid synthesis of <sup>18</sup>F-FMISO and analogs thereof. New precursors such as 1-(2'-nitro-1'-imidazolyl)-2-O-acetyl-3-O-tosylpropanol, glycerol-1,3-ditosylate-2-O-acetylate, 1-(2'-nitro-1'-imidazolyl)-2,3-O-diacetyl-4-O-tosylbutanol and threitol 1,4-di-tosylate-2,3-O-diacetylate, are also important aspects of the invention. A further aspect of the invention is the development of a hydrophilic PET ligand to image tumor hypoxia. Erythrosyl analogue of 2-nitroimidazole (Ts-ETNIM) was prepared from a mixture of 2-nitromidazole, ditosylthreitol and cesium carbonate at 60 °C for 1 hr. Ts-ETNIM was isolated at 70 % yield. [<sup>18</sup>F]fluoroerythronitroimidazole (FETNIM) was then prepared from Ts-ETNIM and K<sup>18</sup>F/kryptofix®. The yield for [<sup>18</sup>F]FETNIM was 26-30 % (60 min, decay corrected). Results of biodistribution and PET studies indicate that [<sup>18</sup>F]FETNIM has the potential to detect tumor hypoxia and is indicated to be less neurotoxic.

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DESCRIPTIONRAPID SYNTHESIS AND USE OF  
<sup>18</sup>F-FLUOROMISONIDAZOLE AND ANALOGSBACKGROUND OF THE INVENTION10 Field of the Invention

Tumor cells are more sensitive to radiation in the presence of oxygen than in its absence Powers et al., 1963). Even a small percentage of hypoxic cells within a tumor could limit response to radiation (Hall, 1988). Hypoxic radioresistance has been demonstrated in many experimental and animal tumors and hypoxia has been directly demonstrated in certain tumor types in man (Moulder et al., 1984; Peters et al., 1982). The occurrence of hypoxia in human tumors has in most cases been inferred from histologic findings and from evidence of hypoxia in animal tumor studies. *In vivo* demonstration of hypoxia has required tissue measurements with oxygen electrodes and the invasiveness of this technique has limited its application. Most attempts to increase the radiosensitivity of tumors by administration of chemical radiosensitizers have not been successful (Gatenby, et al., 1988; Kayama et al., 1991; Maor et al., 1981). However, there has been no clinically applicable means of demonstrating tumor hypoxia and it has not been possible to identify the patients who could potentially benefit from radiosensitizing therapy. Potential advantages of neutrons over more conventional radiation include a lesser dependence on oxygenation of the tumor and a lower variability of cell neutron sensitivity around the cell cycle.

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3 - [ $^{18}\text{F}$ ]Fluoro-1-(2'-nitro-1'-imidazolyl)-2-propanol  
( $^{18}\text{F}$ -fluoromisonidazole; FMISO) has been used with  
positron emission tomography (PET) to differentiate  
metabolically active hypoxic tumor from well-oxygenated  
5 metabolically active tumor.  $^{18}\text{F}$ -FMISO is metabolized by  
intracellular nitroreductases and acts as a competing  
electron acceptor at low oxygen levels. The  $^{18}\text{F}$ FMISO is  
reduced and subsequently incorporated into metabolically  
active hypoxic cells by covalent bonding to various  
10 macromolecules. Recent studies have shown that PET, in  
view of its ability to monitor cell oxygen content  
through  $^{18}\text{F}$ -FMISO, has a high potential to predict  
treatment response. (Koh et al., 1992; Valk et al.,  
1992; Martin et al., 1989; and Rasey et al., 1989.)

15  
Assessment of tumor hypoxia with  $^{18}\text{F}$  FMISO prior to  
radiation therapy provides a rational means of selecting  
patients for treatment with chemical radiosensitizing  
drugs. Such selection of patients permits more accurate  
20 evaluation of radiosensitizing drugs, since their use  
could be limited to patients with hypoxic tumors, who  
could potentially benefit. In addition, it is possible  
to differentiate radiotherapy modalities (neutron versus  
photon radiotherapy) by correlating [ $^{18}\text{F}$ ]FMISO results  
25 with tumor response.

The synthesis of [ $^{18}\text{F}$ ]FMISO reported by others  
showed no resemblance to the present inventive methods.  
Most studies used a two-part, two-step synthetic  
30 procedure, whereas the precursor of the present invention  
has a nitroimidazole moiety. Although there is a great  
demand in PET for [ $^{18}\text{F}$ ]FMISO, a simple and efficient  
synthesis to produce sufficient radioactivity of  
[ $^{18}\text{F}$ ]FMISO is difficult. Prior studies used  
35 [ $^{18}\text{F}$ ]epifluorohydrin to react with 2-nitroimidazole  
(Hwang et al., 1989; Jerabek et al., 1986; Grierson et  
al., 1989). The reaction takes a longer time (90 min.)

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and provides a lower radiochemical yield. One aspect of the present invention concerns a more rapid synthesis of [ $^{18}\text{F}$ ]FMISO.

5           Three classes of agents have found practical use for therapy against hypoxic tumors (Hall, 1994). These are (1) radiosensitizers of hypoxic cells, (2) chemopotential agents and (3) bioreductive drugs. Radiosensitizers are chemical or pharmacologic agents  
10 that increase the lethal effects of radiation when administered in conjunction with it. Nitroimidazoles were reported to potentiate the cytotoxic effects when combined with other chemotherapeutic agents (e.g. cisplatin, bleomycin, cyclophosphamide and nitrosourea).  
15 Several clinical trials are in progress combining a radiosensitizer with alkylating agents. It would increase the efficiency of chemotherapy if the hypoxic component could be identified by a labeled tracer prior to the therapy. Bioreductive drugs are not  
20 radiosensitizers, yet, these drugs are reduced intracellularly in hypoxic cells to form active cytotoxic agents. Three drugs are primarily used in clinical trials. These drugs are mitomycin C, triapazamine (SR4233) and dual-function nitroheterocyclic compounds  
25 (RB6145).

Two nitroimidazole analogues (misonidazole and etanidazole) are potent radiosensitizers. In clinical  
30 trails, patients with high hemoglobin levels and cancer of the pharynx showed a great benefit from the addition of these analogues (Hall, 1994). However, the dose-limiting toxicity was found to be peripheral neuropathy that progressed to central nervous system toxicity if drug administration was not stopped. This neurotoxicity  
35 prevented the use of the drug at adequate dose levels throughout multifraction treatments.

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Therefore, it is necessary to develop a more hydrophilic agent than misonidazole. This would allow the agent to have shorter half-life *in vivo* and, be expected to show less neurotoxicity. Another aspect of the present invention is the synthesis and evaluation of a new 2-nitroimidazole analogue which is more hydrophilic than FMISO and misonidazole. The synthetic scheme is shown in FIG. 6.

10

#### SUMMARY OF THE INVENTION

The present invention includes a first description of new compounds including: 1-(2'-nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol; glycerol-1,3-ditosylate-2-0-acetylate; 1-(2'-nitro-1'-imidazolyl)-2,3-acetyl-4-0-tosylbutanol; and threitol 1,4-di-tosylate-2,3-0-di-acetylate.

20 The present invention also involves a method for preparing 1-(2'-Nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol comprising reacting glycerol-1,3-ditosylate-2-0-acetylate with 2-nitroimidazole. This reaction is preferably in dimethylformamide in combination with cesium carbonate. Preferable conditions for this reaction are at a temperature of 50°C and for a time of about 1 hour. The present invention also involves a method for preparing <sup>18</sup>F-fluoromisonidazole comprising reacting 1-(2'-nitro-1'-imidazolyl)-2-30 propanol with <sup>18</sup>F complexed with 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane or the like. This reacting is preferably in an acetonitrile solvent. Reaction conditions here are preferably from about 90°C to about 100°C.

35

Methods of the present invention include: A method for preparing 1-(2'-nitro-1'-imidazolyl)-2,3-acetyl-4-0-

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tosylbutanol comprising reacting threitol-1,4-di-  
tosylate-2,3-O-acetylate with 2-nitroimidazole. Also  
part of the present invention is a method for preparing a  
18F-fluoromisonidazole butanol analogue useful for PET  
5 imaging and localization of hypoxic tissue. This method  
comprises reacting 1-(2'-nitro-1'-imidazolyl)-2,3-acetyl-  
4-O-tosylbutanol with 18F complexed with 4,7,13,16,21,24-  
hexaaxo-1,10-diazabicyclo(8,8,8)hexacosane or the like.  
The present invention also comprises a method for  
10 preparing 131I-iodomisonidazole comprising reacting 1-  
(2'-nitro-1'-imidazolyl)-2-acetyl-3-O-tosylpropanol with  
a salt of 131I such as Na131I.

Another aspect of the present invention is the  
15 preparation of a more hydrophilic analogue of  
misonidazole, [18F]fluoroerythro-2-nitroimidazole. This  
compound is prepared from a novel precursor, (2'-nitro-  
1'-imidazolyl)-2,3-isopropylidene-4-tosylbutanol.

20 The method for preparing (2'-nitro-1'-imidazolyl)-  
2,3-isopropylidene-4-tosylbutanol comprises reacting 1,4-  
ditosyl-2,3-isopropylidene-D-threitol with 2-  
nitroimidazole, and preferably the reacting is in  
dimethylformamide, and preferably the reacting is in the  
25 presence of cesium carbonate. The reacting is also  
preferably at about 60°C for about 1 hour.

A further aspect of the invention is a method for  
preparing [18F]fluoroerythro-2-nitroimidazole comprising  
30 reacting (2'-nitro-1'-imidazolyl)-2,3-isopropylidene-4-  
tosylbutanol with 18F complexed with 4,7,13,16,21,24-  
hexaaxo-1,10-diazabicyclo[8,8,8]hexacosane. In this  
method, the reacting is preferably in acetonitrile.

35 An embodiment of the present invention is a method  
of use of [18F]fluoroerythro-2-nitroimidazole for  
assessing tumor hypoxia. The method of use preferably

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comprises administering to a patient an effective amount of [ $^{18}\text{F}$ ]fluoroerythro-2-nitroimidazole and subjecting the patient to positron emission tomography or single photon emission computed tomography.

5

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 schematically shows the synthesis of  
10 [ $^{18}\text{F}$ ]fluoromisonidazole and its analogue.

FIG. 2 schematically shows the synthesis of -(2'-nitro-1'-imidazolyl)-2,3-O-acetyl-4-O-tosyl butanol.

15 FIG. 3 shows a PET image of a patient with nasalpharyngeal cancer (NPC) after receiving 10 mCi each of ( $^{18}\text{F}$ )FMISO prepared according to the present invention.

20 FIG. 4 shows a PET image of a second patient with nasalpharyngeal cancer (NPC) after receiving 10 mCi each of ( $^{18}\text{F}$ )FMISO prepared according to the present invention.

FIG. 5 schematically shows the radiosynthesis of  
25 ( $^{131}\text{I}$ )iodomisonidazole.

FIG. 6 schematically shows the synthesis of  
[ $^{18}\text{F}$ ]FETNIM.

30 FIG. 7 shows the PET images of tumor bearing rabbits 1 hour post-injection with [ $^{18}\text{F}$ ]FETNIM.

FIG. 8 shows the autoradiogram and photographic studies of mammary tumor bearing rats treated with  
35 [ $^{18}\text{F}$ ]FETNIM, [ $^{18}\text{F}$ ]FDG and [ $^{18}\text{F}$ ]FMISO.



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FIG. 9 is a histogram showing tumor-to-tissue ratios of [ $^{18}\text{F}$ ]FETNIM in tumor bearing rats.

## 5 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present precursors for the preparation of [ $^{18}\text{F}$ ]FMISO or its butanol analog are of value clinically and scientifically. The precursor for [ $^{18}\text{F}$ ]FMISO is 1-  
10 (2'-nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol. Such a precursor can be radiolabeled in a short time with  $^{18}\text{F}$  ( $t_{1/2}$ =(109.7 min.),  $^{123}\text{I}$  ( $t_{1/2}$ =13.3 hours) or  $^{131}\text{I}$ . The first two radioisotopic ligands are useful to detect hypoxic tumor volume by positron emission tomography  
15 (PET) and single photon emission computed tomography (SPECT).

[ $^{18}\text{F}$ ]FMISO was prepared as schematically shown in FIG. 1. Glycerol-1,3-ditosylate (compound 2) was  
20 obtained by treating glycerol with tosyl chloride according to a previously reported method (Benbouzid et al., 1988). Compound 3 (2-acetyl glycerol-1,3-ditosylate) was prepared by reacting glycerol-1,3-ditosylate with anhydrous acetic anhydride and  
25  $\text{BF}_3 \cdot \text{etherate}$  (yield 100%). In a mixture of compound 3, 2-nitroimidazole and  $\text{Cs}_2\text{CO}_3$  in dry DMF at  $50^\circ\text{C}$ , compound 4 [1-(2'-nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol] was prepared. (When this reaction was carried out at  $100^\circ\text{C}$  for 16 hr, the undesired compound 5 resulted.)  
30 HPLC showed the retention time of compound 4 to be 9.83 minutes (UV 310 nm, C-18 reverse phase column eluted with  $\text{H}_2\text{O}:\text{MeOH}$ ; 0-80% at a flow rate of 1.5 ml/min.

The butanol FMISO analog was analogously prepared.  
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Compound 1 ([<sup>18</sup>F]fluoromisonidazole) is prepared just prior to use by reacting compound 4 with [<sup>18</sup>F]/KRYPTOFIX® 222.

5 Reagents and conditions for the steps in FIG. 1 and FIG. 2: i) TsCl (2eq), Pyridine, 0°C, 48h (90%); ii) Ac<sub>2</sub>O (1.5 eq) BF<sub>3</sub>·Et<sub>2</sub>O (in anhydrous ether, 0°C, 30 min (100%), iii) 2-nitroimidazole, (0.9 eq), Cs<sub>2</sub>CO<sub>3</sub> (0.9 eq), DMF, 50°C, 1h (48%); iv) [<sup>18</sup>F]/KRYPTOFIX®, CH<sub>3</sub>CN, 95°C, 10 min.,  
10 H<sup>+</sup>, 90°C, 5 min. (12-18%).

Accomplished syntheses of misonidazole and misonidazole analogue precursors, <sup>18</sup>F-fluoromisonidazole (FMISO), [<sup>131</sup>I]iodomisonidazole (<sup>131</sup>I-IMISO) and <sup>18</sup>F-  
15 fluoromisonidazole analogues are described in the Examples below.

The synthesis scheme for [<sup>18</sup>F]FETNIM is depicted in FIG. 6. Biodistribution studies of [<sup>18</sup>F]FETNIM were  
20 performed at 1, 2 and 4 hours in mammary tumor-bearing rats (10 µCi, iv, N=3/time interval). Autoradiograms were performed at 1 hour postinjection of [<sup>18</sup>F]FETNIM, and a comparison has been made with [<sup>18</sup>F]fluoromisonidazole (FMISO). Intratumoral oxygen tension measurements were  
25 performed using the Eppendorf computerized histographic system. Twenty to twenty-five pO<sub>2</sub> measurements along each of 2 to 3 linear tracks were performed at 0.4 mm intervals on each tumor. PET imaging studies of [<sup>18</sup>F]FETNIM were performed in rabbits bearing V2 tumors  
30 (N=3, 4 mCi, iv) which were located on the right leg.

Biodistribution of [<sup>18</sup>F]FETNIM at 1, 2 and 4 hours showed tumor/blood count density ratios of 2.29±0.599, 2.41±0.567 and 8.02±2.420; tumor/muscle ratios of  
35 0.66±0.267, 2.11±0.347 and 5.92±2.240, respectively. Bone uptake did not alter significantly. Autoradiograms

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indicated that [ $^{18}\text{F}$ ]FETNIM and [ $^{18}\text{F}$ ]FMISO could differentiate hypoxic versus necrotic regions in the tumor. The tumor oxygen tension, measured by an oxygen needle probe, was 3.2-6.0 mmHg. PET imaging studies indicated that the hypoxic tumor could be visualized at 1 hour postinjection.

The data disclosed herein indicate that [ $^{18}\text{F}$ ]FETNIM and [ $^{18}\text{F}$ ]FMISO are selective to hypoxic cells. However, [ $^{18}\text{F}$ ]FETNIM appears to be more hydrophilic than [ $^{18}\text{F}$ ]FMISO as evidenced from the autoradiogram and chemistry. Therefore, [ $^{18}\text{F}$ ]FETNIM has the potential to be used as a diagnostic tool and as a follow-up for conventional therapy or chemotherapy of hypoxic tumors. Other useful applications of this compound would be in the diagnosis of brain ischemia (acute stroke), nasopharyngeal cancer, inflammation and cardiac infarction. Additionally, because of the selectivity and specificity of [ $^{18}\text{F}$ ]FETNIM, labeling erythronitroimidazole with a high dose of  $^{131}\text{I}$  would allow for radionuclide therapy of hypoxic tumors.

In summary, a simple and efficient synthetic method to produce sufficient radioactivity of [ $^{18}\text{F}$ ]fluoroerythronitroimidazole, a new PET agent for imaging tumor hypoxia, has been prepared. PET imaging studies, autoradiograms and biodistribution of this compound indicate that [ $^{18}\text{F}$ ]fluoroerythronitroimidazole is selective to hypoxic cells, chemically stable and more hydrophilic than misonidazole. It is thus a useful compound for the evaluation of hypoxic tumors. The diagnostic information obtained from [ $^{18}\text{F}$ ]FETNIM would be important for the follow-up therapy of patients with hypoxic tumors.

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EXAMPLE 1Experimental Section (Applying to these Examples)

5 Nuclear magnetic resonance (NMR) spectra ( $^1\text{H}$  and  $^{13}\text{C}$ ) were recorded at ambient temperature on an IBM-Bruker Model NR/200 AF spectrometer in the Fourier transform mode in  $\text{CDCl}_3$  with tetramethylsilane as an internal reference. Chemical shifts ( $\delta$ ) are reported in parts per million (ppm) and coupling constants (J) in  
10 hertz. Mass spectral analyses were conducted at the University of Texas, Health Science Center at Houston, Texas. The mass data was obtained by fast-atom bombardment on a Kratos MS50 instrument. The elemental analyses were conducted at Galbraith Laboratories Inc.  
15 (Knoxville, TN). High resolution mass spectroscopy (HRMS) was performed at the Midwest Center for Mass Spectrometry (Lincoln, NE). All chemical reactions were conducted in dry glassware and were protected from atmospheric moisture. Solvents were dried over freshly  
20 activated ( $300^\circ\text{C}$ , 1h) molecular sieves (type 4A). The homogeneity of the products was determined by ascending thin-layer chromatography (TLC) on silica-coated glass plates (silica gel 60 F 254, Merck) with mixtures of  $\text{CHCl}_3$ -MeOH as the eluting solvent. Preparative  
25 separations were performed by column chromatography on silica gel (Merck, 230-400 mesh) with mixtures of  $\text{CHCl}_3$ -MeOH as eluant.

EXAMPLE 2Synthesis of glycerol-1,3-ditosylate (Compound 2)

30 Compound 2 was synthesized according to the previous reported method with modifications (Benbouzid et al., 1988). p-Toluenesulfonyl chloride (10.29 g, 54 mmol) dissolved in dry pyridine was added to a stirred solution  
35 of anhydrous glycerol (2.48 g, 27 mmol) in dry pyridine (30 ml at  $0^\circ\text{C}$ ). the solution was added slowly and left

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to react for 44 hours in the refrigerator (0-3°C). The pink mixture was poured over crushed ice and acidified with concentrated HCl. The organic layer was separated and the aqueous layer was washed with methylene chloride (2 x 50 ml). The combined organic extracts were washed successively with 2N HCl (2 x 10 ml) and distilled water (2 x 10 ml) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The filtrate was evaporated and the residual crude product was purified by column chromatography on silica gel to afford Compound 2 as an oil: 9.72 g (24.3 mmol) yield 90%. <sup>1</sup>H NMR [Chemical shift (δ), multiplicity, coupling constant (Hz), number of protons, atom]: 7.75 (d; J=8.2 Hz; 4H, Har.); 7.35 (d; J=8.2 Hz; 4H; Har.); 4.1 (bs; 4H; H<sub>1,3</sub>); 3.35 (bs; 1H; H<sub>2</sub>); 2.4 (s; 6H; 2CH<sub>3</sub>). <sup>13</sup>C NMR (ppm): 145 (C ar.); 131.7 (C ar.); 129.7 (CH ar.); 127.6 (CH ar.); 69.3 (C<sub>1,3</sub>); 66.7 (C<sub>2</sub>); 21.2 (CH<sub>3</sub>). Satisfactory <sup>1</sup>H and <sup>13</sup>C NMR data as well as mass spectral data were recorded for this compound. These spectra were consistent with the structures shown in FIG. 1.

### EXAMPLE 3

#### Synthesis of Glycerol-1,3-ditosylate-2-0-acetylate (compound 3)

Glycerol-1,3-ditosylate (5 g, 12.5 mmol) was added dropwise, while stirring 5 minutes at about 0°C, to a solution of acetic anhydride (2 ml, 20 mmol) and BF<sub>3</sub>·etherate (1.0 ml) in anhydrous ether (50 ml). The reaction mixture was stirred for 10 minutes, washed successively with 25% sodium acetate solution (10 ml) and water (2 x 15 ml), and then dried over anhydrous sodium sulfate. The solvent was evaporated to yield glycerol-1,3-ditosylate-2-0-acetylate as a white solid (5.48 g, 12.4 mmol). The structure of the product was confirmed by <sup>1</sup>H-NMR <sup>13</sup>C-NMR, mass spectral data and elemental analysis. These spectra were consistent with the structure of glycerol-1,3-ditosylate-2-0-acetylate shown

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in FIG. 1.  $^1\text{H}$  NMR: 7.8 (d;  $J=8.0$  Hz; 4H; Har.); 7.3 (d;  $J=8.0$  Hz; 4H; Har.); 5.05 (t;  $J=4.8$  Hz; 1H;  $\text{H}_2$ ); 4.1 (d;  $J=4.8$  Hz; 4H;  $\text{H}_{1,3}$ ); 2.4 (s; 6H;  $2\text{CH}_3\text{ar.}$ ); 1.8 (s; 3H;  $\text{CH}_3\text{ac.}$ ).  $^{13}\text{C}$  NMR (ppm): 169.6 ( $\text{COCH}_3$ ); 145.3 (C ar.); 132.2 (C ar.); 130 ( $\text{CH ar.}$ ); 127.9 ( $\text{CHar.}$ ); 68.1 ( $\text{C}_2$ ); 66.5 ( $\text{C}_{1,3}$ ); 21.6 ( $\text{CH}_3\text{ ar.}$ ); 20.5 ( $\text{OCOCH}_3$ ). mass [FAB,  $(\text{C}_{19}\text{H}_{22}\text{S}_2\text{O}_8)^+$ ]  $m/z$ : 441 ( $\text{M}^+$ , 5%); 383 ( $\text{M}^+ - \text{OCOCH}_3$ , 4%); 271 ( $\text{M}^+ - \text{tosylate}$ , 100%), 229 ( $\text{M}^+ + 1 - \text{tosylate} - \text{COCH}_3$ ; 3%).  
Anal. Calc. ( $\text{C}_{19}\text{H}_{22}\text{S}_2\text{O}_8$ ): C: 51.58, H:4.97 Found: C:51.50  
H:4.93.

#### EXAMPLE 4

##### Synthesis of 1-(2'-Nitro-1'-imidazolyl)- 2-0-acetyl-3-0-tosylpropanol (Compound 4)

15

A mixture of glycerol-1,3-ditosylate-2-0-acetylate (0.44 g, 1 mmol), 2-nitroimidazole (0.1 g, 0.9 mmol) and cesium carbonate (0.29 g, 0.9 mmol) in 10 ml of dry DMF, through which argon was bubbled for 10 minutes. The mixture was heated at  $50^\circ\text{C}$  for 1 hour. The mixture was then cooled and DMF was carefully removed under reduced pressure. The residue was taken up in ethyl acetate and filtered. Removal of ethyl acetate *in vacuo* gave a yellow oil which was chromatographed on silica gel eluted with 50-67% ethyl acetate/petroleum ether to afford 1-(2'-Nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol as a white solid (0.17 g, 0.43 mmol), yield 48%. This was dried *in vacuo* and stored at  $0^\circ\text{C}$ . The structure of compound 4 was confirmed by  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR, and mass spectral data. The spectra were consistent with the structure of 1-(2'-nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol shown in FIG. 1. HPLC showed the retention time of this compound to be 9.83 minutes (UV 310 nm, C-18 reverse phase column, eluted with water/methanol 0-80% at a flow rate of 1.5 ml/min). However, if the reaction was heated at  $100^\circ\text{C}$  for 16 hours, a major byproduct (Compound 5) was isolated.

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Compound 4: IR (Nujol) ( $\text{cm}^{-1}$ ): 1760; 1610; 1560; 1500; 1480; 1300; 1240; 1200.  $^1\text{H}$  NMR: 7.8 (d; 2H;  $J=8.1$  Hz; 2Har), 7.4 (d; 2H;  $J=8.1$  Hz; 2Har), 7.1 (d; 2H;  $J=2.9$ ; H-imidazolyl), 5.3 (m; 1H;  $\text{H}_2$ ), 4.85 (dd; 1H;  $J=14.4$ , 3.5 Hz;  $\text{H}_{3a}$ ), 4.5 (dd; 1H;  $J=14.4$ , 8.4 Hz;  $\text{H}_{3b}$ ), 4.2 (dd; 2H;  $J=5, 4.1$  Hz;  $\text{H}_1$ ), 2.45 (s; 3H;  $\text{CH}_3\text{tosyl}$ ), 1.95 (s; 3H;  $\text{CH}_3\text{ac}$ ).  $^{13}\text{C}$  NMR (50 MHz),  $\delta$ (ppm): 169.3 (C=O), 145.6 (Car), 132 (Car), 130.1 (CHar), 129.9 (C-imidazolyl), 128.3 (CH-imidazolyl), 127.9 (CHar), 126.5 (CH-imidazolyl), 68.8 ( $\text{C}_2$ ), 67.2 ( $\text{C}_1$ ), 49.3 ( $\text{C}_3$ ), 21.6 ( $\text{CH}_3\text{tosyl}$ ), 20.3 ( $\text{CH}_3\text{ac}$ ). HRMS calc. for  $\text{C}_{15}\text{H}_{18}\text{N}_3\text{SO}_7$  384.08655, Found 384.0869.

Compound 5:  $^1\text{H}$ NMR: 7.15 (2H, H-imidazolyl); 4.9 (s, 1H, H-imidazolyl); 4.8 (m, 1H,  $\text{H}_2$ ); 4.2 (d, 4H,  $J=3.5$  Hz;  $\text{H}_{1,3}$ ), 2.1 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$ NMR; 171.1 (C=O), 127.7 (CH-imidazolyl); 68.2 (CH- $\text{NO}_2$ ); 65.4 ( $\text{C}_2$ ); 52.8 ( $\text{C}_{1,3}$ ), 21 ( $\text{CH}_3$  ac). HRMS calcd for  $\text{C}_8\text{H}_{12}\text{N}_3\text{O}_4$  214.08278, found 214.0827.

#### EXAMPLE 5

##### Radiosynthesis of [ $^{18}\text{F}$ ]FMISO (Compound 1)

[ $^{18}\text{F}$ ]Fluoride was obtained from the cyclotron facility of the University of Texas M.D. Anderson Cancer Center as produced by proton irradiation of enriched [ $^{18}\text{O}$ ]water in a small-volume silver target. An aliquot containing 250-500 mCi of  $^{18}\text{F}$  activity was combined with 26 mg of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane (KRYPTOFIX® 222) and anhydrous potassium carbonate (4.6 mg), heated under reduced pressure to remove [ $^{18}\text{O}$ ]water, and dried by azeotropic distillation with acetonitrile (3 x 1.5 ml). The tosyl analogue of misonidazole (compound 4) (5 mg), prepared from 1,3-ditosyl-2-O-acetyl glycerol (compound 3) was dissolved in acetonitrile (1.5 ml), and the KRYPTOFIX® 222-fluoride complex added. The mixture was

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then warmed at 95°C for 10 min. After cooling, the reaction mixture was passed through a silica gel Sep-Pak column (Whatman Lab, Clifton, NJ) and eluted with ether (2 x 2.5 ml). The solvent was evaporated and the

5 resulting mixture hydrolyzed with 1N HCl (2 ml) at 90°C for 5 min. The resultant mixture was cooled under N<sub>2</sub> and neutralized with 2N NaOH (0.8 ml) and 1N NaHCO<sub>3</sub> (1 ml). The mixture was then processed by passage through a short

10 alumina C-18 (Sep-Pak) column and a 0.22 µm Millipore filter, followed by 5 ml of 10% ethanol saline. Comparison with an <sup>1</sup>H-NMR spectrum of the unlabeled fluoromisonidazole (FMISO) confirmed the structure. A

15 yield of 20-40 mCi of pure product was isolated (12-18% yield, decay corrected) with end-of-bombardment (EOB) at 60-70 min. HPLC was performed on a C-18 Radial-Pak column, 8 x 100 mm, with pure water, using a flow rate of

20 1 ml/min. The no-carrier-added product corresponded to the retention time (4.8 min) of the unlabeled fluoromisonidazole under similar conditions. The radiochemical purity was greater than 95%. Under UV

25 detection (310 nm), there are no other impurities. A radio-TLC scanner (Bioscan, Washington, DC) showed the retardation factor of 0.5 for the final product, using a silica gel plate G/UV 254, 5 x 20 cm (Whatman, Anaspec, MI) chloroform: methanol (7:3) eluant, which corresponded

30 to unlabeled FMISO. In addition, KRYPTOFIX® 222 was not visualized (developed in iodine chamber) on the silica gel-coated plate using 0.1% v/v triethylamine in methanol as an eluant. The specific activity of FMISO was

determined to be in the range of 1-2 Ci/µmol based upon UV and radioactivity detection of a sample of known mass and radioactivity. The identity and purity of [<sup>18</sup>F]FMISO were confirmed by HPLC.



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EXAMPLE 6Half-life Test of [ $^{18}\text{F}$ ]fluoromisonidazole

After the [ $^{18}\text{F}$ ]fluoromisonidazole was prepared, an  
5 aliquot (10  $\mu\text{Ci}$ ) of this radioactive product was counted  
on a gamma counter at various time intervals (30, 60, 120  
min.). To assure the correct isotope ( $^{18}\text{F}$ ) is  
incorporated into the nitroimidazole analogue, the  
radioactivity of the final product at different time  
10 intervals is counted and decay is corrected.

EXAMPLE 7Sterility and Pyrogenicity

15 To demonstrate sterility, each batch of product is  
tested using Bactec culture vials with aerobic and  
anaerobic materials (NR6 and NR7) (Towson, MD). An  
aliquot (0.3 ml) of the final solution is incubated in  
vials for 7 days at 37°C. Sterility is examined every  
20 day and assayed by visualizing the cloudiness of the  
solution. The ten samples tested have consistently been  
shown to be sterile.

To assure low or absent pyrogenicity, a LAL kit  
25 (Whittaker Bioproduct, Walkersville, MD) is used. To  
prove drug solution does not enhance or inhibit the LAL  
test, we used three 1:1 dilutions (0.25 ml of drug  
solution to 0.25 ml of sterile water) to test  
pyrogenicity of each batch of product. Compared to  
30 standard solutions (12.5  $\text{E}\mu/\text{ml}$ , 1.25  $\text{E}\mu/\text{ml}$  and negative),  
the tested product solutions (ten samples) proved to be  
pyrogen-free (0  $\text{E}\mu/\text{ml}$ ) and did not interfere with the  
assay sensitivity.

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EXAMPLE 8Pharmacology and Toxicology Data**Pharmacology**

5

Fluoromisonidazole (FMISO) has been shown to bind selectively to hypoxic cells *in vitro* and *in vivo* at radiobiologically significant oxygen levels. In patient studies, the hypoxic elements within a tumor volume was defined as regions with a threshold regional tumor to plasma ( $^{18}\text{F}$ -FMISO) ratio of  $\geq 1.4$  by 2 or more hours after injection.

The following Table summarizes the results of dosimetry calculation for radiation exposure to several hormonal tissues.

20

Dosimetry:	Normal Tissue Dosimetry for $^{18}\text{F}$ -FMISO	
Organ	mrad/mCi $\pm$ SD	7 mCi/70 Kg
Liver	26.8 $\pm$ 9.7	190 mrad (n=3)
Kidney	25.8 $\pm$ 5.5	180 mrad (n=3)
Muscle	16.8 $\pm$ 7.9	120 mrad (n=6)
Bladder	81.5 $\pm$ 47	570 mrad (n=2)

Muscle is typical of most normal tissues; the total body musculature receives 120 mrem in a routine study for a 70 Kg male given the dose of 0.1 mCi/Kg. The liver and kidneys receive nearly twice the muscle dose, and the bladder wall about five times as much.

30

The data obtained from this study demonstrate the feasibility of using  $^{18}\text{F}$ -FMISO to detect hypoxia in human head and neck tumors. Clinical trials will determine whether a relationship exists between  $^{18}\text{F}$ -FMISO uptake and tumor radiation response.

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### Toxicity

Nitroimidazoles are chemotherapeutically important as antiprotozoal and antibacterial agents (Hoffer et al.). The most common side effect of MISO toxicity is peripheral sensory neuropathy of the hands and feet, characterized by mild to moderate paresthesias and loss of vibration sensitivity (McNeill et al.). Others have reported convulsions after large single doses, and the development of toxicity, and encephalopathies, following small multiple doses (McNeill et al.). The dose which affects the neurotransmitter system was in the range of 0.76 mg/g (in mice).

The specific activity of  $^{18}\text{F}$ -FMISO prepared is in the range of 1-3 Ci/ $\mu\text{mol}$ . The physical material will be in the range of 2-6  $\mu\text{g}$  per patient. This radiotracer dose is far below the toxicity dose. In addition, animal acute toxicity studies have been performed, rats (n=3) were administered 2mCi/Kg as a single dose. All rats tolerated this dose without toxicity on longer follow-up periods (5 days). This dose is ten-fold higher than the dose thus far studied.

25

#### EXAMPLE 9

#### PET Imaging Studies Using [ $^{18}\text{F}$ ]FMISO

PET imaging studies were performed on two patients with nasopharyngeal cancer (NPC). Each patient was positioned supine in the scanner so that the detector rings would span the entire tumor region. A 20-min attenuation scan was performed with a 4 mCi  $^{68}\text{Ge}$ -ring source prior to tracer injection. After each patient had received 10 mCi of [ $^{18}\text{F}$ ]FMISO, eight consecutive 15-min scans were taken. The total number of counts collected per scan was in the range of 15-30 million. Each patient

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was also administered [ $^{18}\text{F}$ ]FDG to ascertain the tumor region.

In the first patient, [ $^{18}\text{F}$ ]FMISO showed tumor uptake in the lymph node region (see FIG. 3) suggesting the tumor metastasized to lymph node is hypoxic. In the second patient, the recurrent primary tumor is hypoxic (see FIG. 4). This study demonstrates that [ $^{18}\text{F}$ ]FMISO is able to detect primary or metastatic hypoxic tumors.

#### EXAMPLE 10

#### Synthesis of L-Threitol-1,4-di- p-tosylate-2,3-O-diacetate (Compound 6)

A solution of L-threitol 1,4-di-p-tosylate (1g, 2.3 mmole) was added dropwise with stirring over 5 min. at room temperature, to a solution of acetic anhydride (3 ml, 31 mmole) and  $\text{BF}_3$ , etherate (0.2 ml) in anhydrous ether (20 ml). The reaction mixture was stirred for 10 min., washed successively with 25% sodium acetate solution (10 ml) and water (25 ml x 2), and dried over anhydrous sodium sulfate. The solvent was evaporated, and the residue was distilled under reduced pressure to give the acetylated compound. Yield 94%. The structure of the desired compound was confirmed by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and mass spectral data.  $^1\text{H}$  NMR: 7.75 (d; J=8.3 Hz; 4H; Har.); 7.35 (d; J=8.3 Hz; 4H; Har.); 5.2 (t; J=3.2 Hz; 2H; H<sub>2</sub>, 3H); 4.2 (t; J=5 Hz; 4H; H<sub>1,4</sub>); 2.5 (s; 6H; 2CH<sub>3</sub>ar.); 2.0 (s; 6H; 2CH<sub>3</sub>ac.).  $^{13}\text{C}$  NMR: 169.5 (OCOCH<sub>3</sub>); 145.3 (C ar.); 132.2 (C ar.); 129.9 (CH ar.); 127.9 (Ch ar.); 68.3 (C<sub>2,3</sub>); 66.4 (C<sub>1,4</sub>); 21.6 (CH<sub>3</sub> ar.); 20.4 (OCOCH<sub>3</sub>). MS [FAB, C<sub>22</sub>H<sub>26</sub>S<sub>2</sub>O<sub>10</sub>] m/z: 515 (M<sup>+</sup>+1, 55); 455 (M<sup>+</sup>-OCOCH<sub>3</sub>, 8%); 342 (M<sup>+</sup>-tosylate, 100%).

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EXAMPLE 11Synthesis of 1-(2'-Nitro-1'-imidazolyl)-2,3  
-O-acetyl-4-O-tosylbutanol  
(Compound 8)

5

2-Nitroimidazole (100 mg, 0.88 mmole) was dissolved in DMF (1 ml), cesium carbonate (289 mg) was added and the reaction stirred for 5 min at room temperature. Threitol 1,4-di-p-tosylate 2,3-diacetate (1.1 eq., 431.5 mg, 0.97 mmole) was added last, and the reaction was stirred at 80°C for 1 hour. The reaction was filtered and the DMF removed *in vacuo*. The residue was chromatographed on alumina eluted with ethyl acetate/petroleum ether. The structure of resultant 1-(2'-Nitro-1'-imidazolyl)-2,3-O-acetyl-4-O-tosylbutanol, the fluoromisonidazole butanol analog, was confirmed by <sup>1</sup>H NMR and <sup>13</sup>C NMR. <sup>1</sup>H NMR: 7.85(d; J=8.2 Hz; 2Har.); 7.4(d; 2H; J=8.2 Hz; 2Har.); 7.15(d; 2H; J=2.9; H-imidazolyl); 5.4 (m; 1H; H2); 5.2 (m; 1H; H3); 4.95(dd; 1H; J=14.5, 3.5 Hz; H4a); 4.5(dd; 1H; J=14.5, 3.5 Hz; H4b) 4.3 (m; 2H; h1); 2.45(s; 3H; CH<sub>3</sub>tosyl); 1.98(s; 6H; 2CH<sub>3</sub>ac.). <sup>13</sup>C NMR 169.4 (OCOCH<sub>3</sub>); 146(Car.); 132(Car.); 130.1(CHAR); 128.3(C-imidazolyl); 127.9(CH-imidazolyl); 127.4(CHAR); 126.7(CH-imidazolyl); 69.3(C2,3); 67.1(C1); 49.3(C4); 21.6(CH<sub>3</sub>tosyl); 20.5(CH<sub>3</sub>ac.).

EXAMPLE 12Synthesis of (<sup>18</sup>F)Fluoromisonidazole butanol analogue  
(Compound 1B)

30

This compound can be prepared from 1-(2'-Nitro-1'-imidazolyl)-2,3-O-acetyl-4-O-tosylbutanol, KRYPTOFIX® 222-fluoride complex in acetonitrile as described for (<sup>18</sup>F)FMISO synthesis. The (<sup>18</sup>F) fluoromisonidazole butanol analog is usable for PET tumor imaging.

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EXAMPLE 13Radiosynthesis of  $^{131}\text{I}$  Iodomisonidazole (Compound 1C)

5 mg of tosylmisonidazole was dissolved in 1 mL of  
5 acetone.  $\text{Na}^{131}\text{I}$  (1 mCi in borate buffer) was added. The  
reaction mixture was heated at 80°C for 1 hour. Radio-  
thin layer chromatographic (TLC) analysis showed two  
peaks with Rf values of 0.01 and 0.72. The first peak  
was free  $\text{Na}^{131}\text{I}$  and the second peak was  $^{131}\text{I}$ -  
10 iodomisonidazole. Radio-TLC indicated the desired  
product has 45% yield. The pure product was obtained  
after hydrolysis and then passed through a silica gel  
column and eluted with ether:petroleum  
ether:triethylamine (1:1:10%). This compound is useful  
15 therapeutically.

EXAMPLE 14Synthesis of (2'-nitro-1'-imidazolyl)-  
2,3-isopropylidene-4-tosylbutanol (Compound 10)

20 A mixture of 1,4-ditosyl-2,3-isopropylidene-D-  
threitol (ET) (0.47 g, 1 mmol), 2-nitroimidazole (0.1 g,  
0.9 mmol), and cesium carbonate (0.29 g, 0.9 mmol) in 10  
ml of dry dimethylformamide (DMF) was heated at 60°C for  
25 1 h. The reaction was then cooled and DMF was removed  
under reduced pressure. The residue was taken up in  
ethyl acetate and filtered. Removal of ethyl acetate in  
vacuo produced a yellow oil which was chromatographed on  
silica gel, eluted with petroleum ether/ethyl acetate  
30 (gradient from 10% to 70%) to afford a white solid (0.27  
g, 0.63 mmol, 70% yield). The structure of the product  
was determined by  $^1\text{H}$  and  $^{13}\text{C}$  NMR and mass spectral data.  
HPLC showed the retention times of the title compound to  
be 12.45 min, and the ET compound to be 14.8 min (UV 254  
35 nm, Brownlee C-18 5 $\mu$  reverse phase column, 4.6x100 mm  
eluted with water/methanol, 0-80%, at a flow rate of 1.0  
ml/min).

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EXAMPLE 15Radiosynthesis of [ $^{18}\text{F}$ ]fluoroerythro-2-nitroimidazole  
(FETNIM) (COMPOUND 9)

5            [ $^{18}\text{F}$ ]Fluoride was produced in the cyclotron facility  
            (42 MeV, Cyclotron Corp., Berkeley, California) at the  
            University of Texas M.D. Anderson Cancer Center by proton  
            irradiation of enriched  $^{18}\text{O}$ -water in a small-volume  
10            titanium target. The target was filled with 2 ml of  
            [ $^{18}\text{O}$ ] water. Aliquots containing 400-500 mCi of  $^{18}\text{F}$   
            activity after 1 hour beam time (12  $\mu\text{A}$  current) was  
            combined with KRYPTOFIX<sup>®</sup>-2,2,2 (26 mg) and anhydrous  
            potassium carbonate (4.6 mg), heated under reduced  
            pressure to remove [ $^{18}\text{O}$ ] water, and dried by azeotropic  
15            distillation with acetonitrile (3x1.5 mL). The tosyl  
            analogue of 2-nitroimidazole (Compound 10, 20 mg),  
            prepared from (Compound 10) was dissolved in acetonitrile  
            (1.5 mL), added to the KRYPTOFIX<sup>®</sup>-fluoride complex, and  
            then warmed at 95°C for 10 min. After cooling, the  
20            reaction mixture was passed through a silica gel Sep-pak  
            column (Whatman Lab, Clifton, NJ) and eluted with ether  
            (2 x 2.5 mL). The solvent was evaporated and the  
            resulting mixture was hydrolyzed with 2 N HCl (1 mL) at  
            105°C for 10 min. The mixture was cooled under  $\text{N}_2$  and  
25            neutralized with 2 N NaOH (0.8 mL) and 1 N  $\text{NaHCO}_3$  (1 mL).  
            The mixture was passed through a short alumina column, a  
            C-18 Sep-Pak column, and a 0.22- $\mu\text{m}$  Millipore filter,  
            followed by eluting 6 mL of 10% ethanol/saline. A yield  
            of 70-80 mCi of pure product was isolated (20-30% yield,  
30            decay corrected) with the end of bombardment (EOB) at 70  
            min. HPLC was performed on a Brownlee (5 $\mu$  reverse phase)  
            column, 4.6x100 mm, with water/methanol, 0-80%, using a  
            flow rate of 1 mL/min. The no-carrier-added product  
            corresponded to the retention time (3.11 min) of the  
35            unlabeled FETNIM under similar conditions. The retention  
            time before hydrolysis of compound 9 was 8.94 min. The  
            radiochemical purity was greater than 99%. Under the UV

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detector (254 nm), there were no other impurities. A radio-TLC scanner (Bioscan, Washington, DC) showed a retardation factor of 0.3 for the final product using a silica gel plate G/UV 254, 5 x 20 cm (Whatman, Anaspec, MI), eluted with chloroform:methanol (7:3), which corresponds to the unlabeled FETNIM. In addition, KRYPTOFIX®-2,2,2 was not visualized (developed in iodine chamber) on the silica gel-coated plate using 0.1% (v/v) triethylamine in methanol as an eluant. The specific activity of [ $^{18}\text{F}$ ]FETNIM ranged from 1 to 2 Ci/ $\mu\text{mol}$  based upon UV and radioactivity detection of a sample of known mass and radioactivity.

#### EXAMPLE 16

#### PET Imaging Studies Using [ $^{18}\text{F}$ ]FETNIM

PET imaging was performed with a positron camera (Positron Corporation, Houston, TX). The tomograph has a field-of-view of 42 cm on transverse and 12 cm on coronal planes. The axial resolution on the reconstructed plane is 1.2 cm. Twenty-one transaxial slices separated by 5.2 mm were reconstructed and displayed in standard uptake value (SUV) which measures the ratio of tissue radiotracer uptake to that of the whole body uptake for each scan.

Three male New Zealand white rabbits each weighing 3 kg were inoculated at a single site in the right buttock area with a 0.5 ml suspension of minced VX2 tumor fragments ( $10^6$  cells/rabbit). The tumors are maintained through serial animal passage and are available from the Department of Veterinary Medicine (The University of Texas M.D. Anderson Cancer Center, Houston, TX). When the tumor size reached 2 cm by two weeks after inoculation, each rabbit was administered 4 mCi of [ $^{18}\text{F}$ ]FETNIM. A 20 minute attenuation scan was performed with a 4 mCi  $^{68}\text{Ge}$ -ring source prior to tracer injection.



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Each rabbit was supine in the scanner so that the detector rings would span the entire lumbar region. Eight consecutive 10 minute scans were acquired. There was a 5 minute wait between scans for data transfer. The  
5 total number of counts collected per scan was in the range of 3-6 million.

The coronal, transaxial and sagittal views of a PET image of a tumor-bearing rabbit 1 hour after  
10 administration of [ $^{18}\text{F}$ ]FETNIM is shown in FIG. 7. The rabbit was scanned from cranial to caudal direction. The tumor can be visualized at one hour postinjection of [ $^{18}\text{F}$ ]FETNIM. The SUV value of the tumor was greater than  
10.

15

#### EXAMPLE 17

#### Autoradiographic studies of [ $^{18}\text{F}$ ]FETNIM in tumor-bearing rats

20 Female Fischer 344 mammary tumor-bearing rats (n=3) after receiving [ $^{18}\text{F}$ ]FETNIM (2 mCi, iv) were sacrificed at 1 h. To ascertain the metabolic and hypoxic character of the tumor, one rat was administered  
[ $^{18}\text{F}$ ]fluorodeoxyglucose and another rat was given  
25 [ $^{18}\text{F}$ ]fluoromisonidazole (2 mCi, iv). The rats were sacrificed at 1 hour postinjection. The rat body was fixed in a carboxymethyl cellulose (4%) block. The frozen body in a block was mounted to a cryostat (LKB 2250 cryo-microtome, Ijamsville, MD) and 40  $\mu\text{m}$  coronal  
30 sections were made. The section was freeze dried, then mounted on a x-ray film (X-Omat AR, Kodak, Rochester, NY) for 24 hours.

35 *In vivo* autoradiographic studies in mammary tumor-bearing rats indicated that the tumor could be visualized 1 hour postinjection. However, in the center region of the tumor, there was a lack of uptake of [ $^{18}\text{F}$ ]FETNIM,

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suggesting tissue necrosis (FIG. 8). The necrotic region can be verified from the photograph. The autoradiogram of [ $^{18}\text{F}$ ]FDG indicated that the tumor was metabolically active. The distribution of [ $^{18}\text{F}$ ]FDG in a tumor-bearing rat was different from [ $^{18}\text{F}$ ]FETNIM and [ $^{18}\text{F}$ ]FMISO. The intestines and colon showed high uptake in [ $^{18}\text{F}$ ]FETNIM and [ $^{18}\text{F}$ ]FMISO. However, the medullary of the kidney area had higher uptake in the [ $^{18}\text{F}$ ]FETNIM specimen.

#### EXAMPLE 18

##### Polar Graphic Oxygen Needle Probe Measurements

To confirm hypoxic tumors detected by imaging, intratumoral  $\text{pO}_2$  measurements were performed using the Eppendorf computerized histographic system. Twenty to twenty-five  $\text{pO}_2$  measurements along each of two to three linear tracks were performed at 0.4 mm intervals on each tumor (40-75 measurements in total). Tumor  $\text{pO}_2$  measurements were conducted on 3 tumor-bearing rats and rabbits. Using an on-line computer system, the  $\text{pO}_2$  measurements of each track were expressed as absolute values relative to the location of the measuring point along the track, and as the relative frequencies within a  $\text{pO}_2$  histogram between 0 and 100 mmHg with a class width of 2.5 mm.

Intratumoral  $\text{pO}_2$  measurement of the mammary tumors and VX2 tumors indicated that both tumor oxygen tensions ranged from 3.2 to 6.0 mmHg; the normal muscle oxygen tension was 30-40 mmHg. Both tumors are hypervascular in their viable region and hypovascular in their central region. Both tumor-bearing animal models are suitable for the evaluation of tumor hypoxia.

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EXAMPLE 19In Vivo Biodistribution of [<sup>18</sup>F]FETNIM  
in Tumor-Bearing Rats

5           Female Fischer 344 rats (250-275 g) (Harlan, Inc., Indianapolis, IN) were inoculated with mammary tumor cells in the lumbar area using a 13762 tumor cell line (s.c. 10<sup>5</sup> cells/rat). After 14 days, a tumor size of 1-2 cm was observed. Three groups of rats (N=3/group) were  
10           anesthetized with ketamine (10-15 mg/rat). The <sup>18</sup>F-FETNIM reconstituted in 5% ethanol/saline was given to rats (10 μCi/rat, iv) and tissue distribution was conducted at 1h, 2h and 4h intervals. The tissues were excised, weighed and counted for radioactivity. The percent of injected  
15           dose per gram of tissue weight was determined.

          The tissue distribution of [<sup>18</sup>F]FETNIM in the tumor-bearing rats is shown in Table 2. Bone had high affinity for ionic fluoride, the bone uptake value did  
20           not alter, suggesting the *in vivo* stability of [<sup>18</sup>F]FETNIM. The tumor/muscle, tumor/blood and tumor/liver count density ratios showed increases with time (FIG. 9), the tumor-to-blood count density being 8.0±2.42 at 4 hour postinjection of [<sup>18</sup>F]FETNIM. This  
25           increased tumor-to-background ratio suggests that [<sup>18</sup>F]FETNIM has the potential to detect tumor hypoxia.

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TABLE 2  
Biodistribution of [ $^{18}\text{F}$ ]Fluoroerythronitroimidazole in Tumor-Bearing Rats<sup>1</sup>  
(Percent of Injected Dose/Gram Weight; N=3/Time Interval)

TIME	1 HOUR	2 HOUR	4 HOUR
BLOOD	0.3493 $\pm$ 0.02173	0.1950 $\pm$ 0.02828	0.0993 $\pm$ 0.01266
LUNG	0.3577 $\pm$ 0.00723	0.2107 $\pm$ 0.05713	0.1027 $\pm$ 0.02122
LIVER	0.4530 $\pm$ 0.01493	0.3123 $\pm$ 0.08225	0.1843 $\pm$ 0.02458
SPLEEN	0.3183 $\pm$ 0.02011	0.2013 $\pm$ 0.05918	0.0953 $\pm$ 0.01401
KIDNEY	0.8860 $\pm$ 0.00424	0.5145 $\pm$ 0.02334	0.4663 $\pm$ 0.06866
BONE	0.2570 $\pm$ 0.14001	0.0810 $\pm$ 0.00424	0.0753 $\pm$ 0.01570
MUSCLE	1.2600 $\pm$ 0.08627	0.2670 $\pm$ 0.09036	0.1377 $\pm$ 0.01893
TUMOR	0.7957 $\pm$ 0.20358	0.5516 $\pm$ 0.15818	0.8113 $\pm$ 0.33773
TUMOR/BLOOD	2.2900 $\pm$ 0.59940	2.4100 $\pm$ 0.56719	8.0200 $\pm$ 2.42000
TUMOR/MUSCLE	0.6602 $\pm$ 0.26659	2.1100 $\pm$ 0.34679	5.9200 $\pm$ 2.24000
TUMOR/LIVER	1.7600 $\pm$ 0.44151	1.7700 $\pm$ 0.25608	4.3300 $\pm$ 1.31000

1. 13762 cell line was inoculated to rats (s.c.  $10^5$  cells/rat). When tumor size reached 1-2cm, each rat was administered  $10\mu\text{Ci}$  tracer.

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REFERENCES

The following references are incorporated by reference herein in pertinent part for the reasons cited  
5 in the above text.

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It is understood that equivalents of the following

35 claims involve simple substitution of analogous chemicals and methods well known to those of skill in the art.

- 30 -

CLAIMS:

1. 1-(2'-Nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol.  
5
2. Glycerol-1,3-ditosylate-2-0-acetylate.
- 10 3. 1-(2'-Nitro-1'-imidazolyl)-2,3-0-diacetyl-4-0-tosylbutanol.
- 15 4. Threitol 1,4-di-tosylate-2,3-0-diacetylate.
5. A method for preparing 1-(2'-nitro-1'-imidazolyl)-2-0-acetyl-3-0-tosylpropanol comprising reacting glycerol-1,3-ditosylate-2-0-acetylate with 2-nitroimidazole.  
20
6. The method of claim 5 where the reacting is in dimethylformamide.
- 25 7. The method of claim 5 where the reacting is in the presence of cesium carbonate.
- 30 8. The method of claim 5 where the reacting is at about 50°C for about 1 hour.
- 35 9. A method for preparing <sup>18</sup>F-fluoromisonidazole comprising reacting 1-(2'-nitro-1'-imidazolyl)-2-



- 31 -

propanol with  $^{18}\text{F}$  complexed with 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane.

5 10. The method of claim 9 where the reacting is in acetonitrile.

10 11. The method of claim 9 where the reacting is at from about  $90^{\circ}\text{C}$  to about  $100^{\circ}\text{C}$ .

12. A method for preparing 1-(2'-nitro-1'-imidazolyl)-2,3-acetyl-4-0-tosylbutanol comprising reacting Threitol-1,4-di-tosylate-2,3-0-acetylate with 2-nitroimidazole.

15

13. A method for preparing a  $^{18}\text{F}$ -fluoromisonidazole butanol analogue comprising reacting 1-(2'-nitro-1'-imidazolyl)-2,3-acetyl-4-0-tosylbutanol with  $^{18}\text{F}$  complexed with 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo(8,8,8)hexacosane.

20

25 14. A method for preparing  $^{131}\text{I}$ -iodomisonidazole comprising reacting 1-(2'-nitro-1'-imidazolyl)-2-acetyl-3-0-tosylpropanol with  $\text{Na}^{131}\text{I}$ .

30 15. (2'-nitro-1'-imidazolyl)-2,3-isopropylidene-4-tosylbutanol.

35 16. [ $^{18}\text{F}$ ]fluoroerythro-2-nitroimidazole.

- 32 -

17. A method for preparing (2'-nitro-1'-imidazolyl)-2,3-isopropylidene-4-tosylbutanol comprising reacting 1,4-ditosyl-2,3-isopropylidene-D-threitol with 2-nitroimidazole.

5

18. The method of claim 17 where the reacting is in dimethylformamide.

10

19. The method of claim 17 wherein the reacting is in the presence of cesium carbonate.

15

20. The method of claim 17 where the reacting is at about 60°C for about 1 hour.

21. A method for preparing [<sup>18</sup>F]fluoroerythro-2-nitroimidazole comprising reacting (2'-nitro-1'-imidazolyl)-2,3-isopropylidene-4-tosylbutanol with <sup>18</sup>F complexed with 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane.

25

22. The method of claim 21 wherein the reacting is in acetonitrile.

30

23. [<sup>18</sup>F]fluoroerythro-2-nitroimidazole for use in assessing tumor hypoxia.

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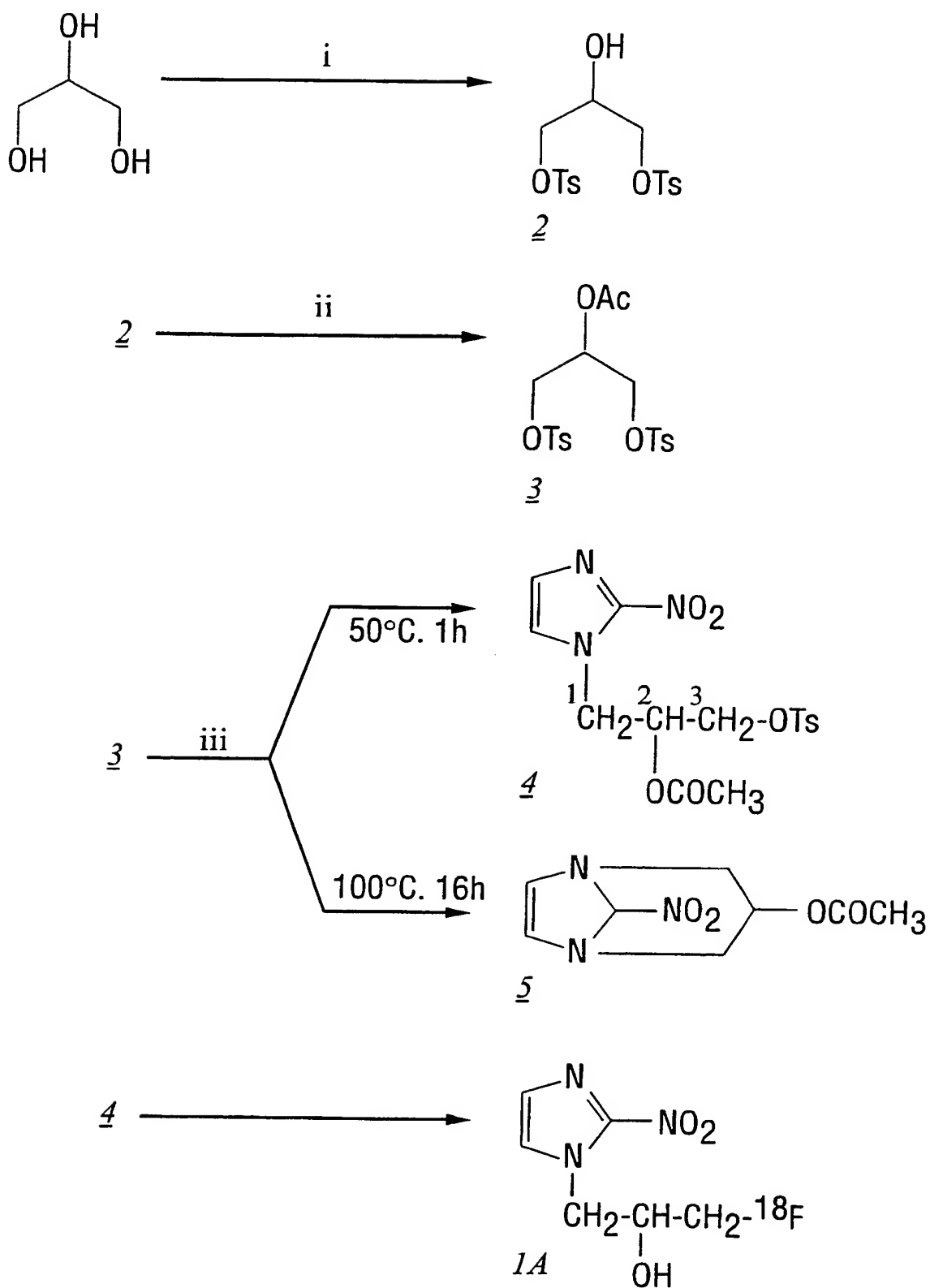


FIG. 1

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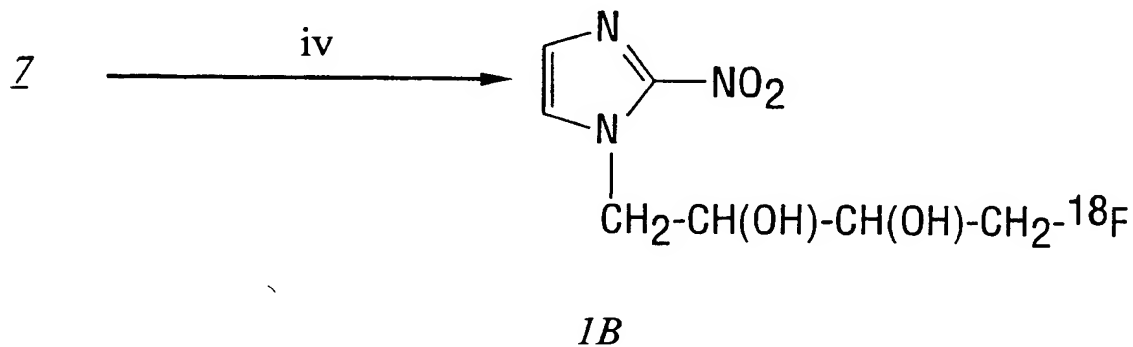
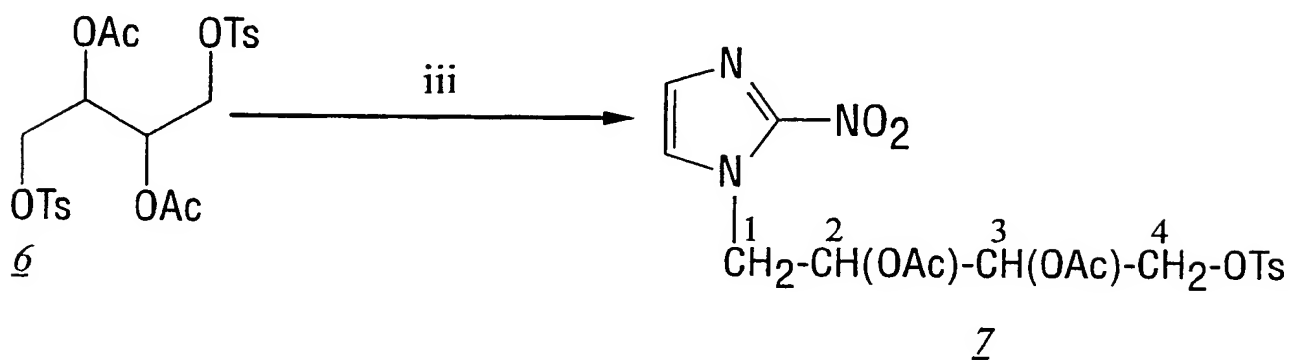
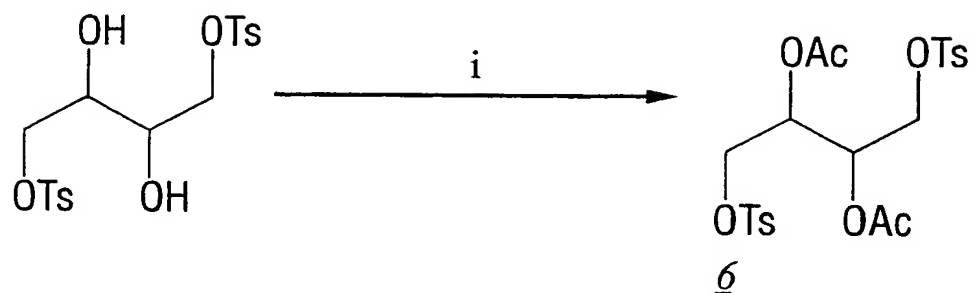


FIG. 2

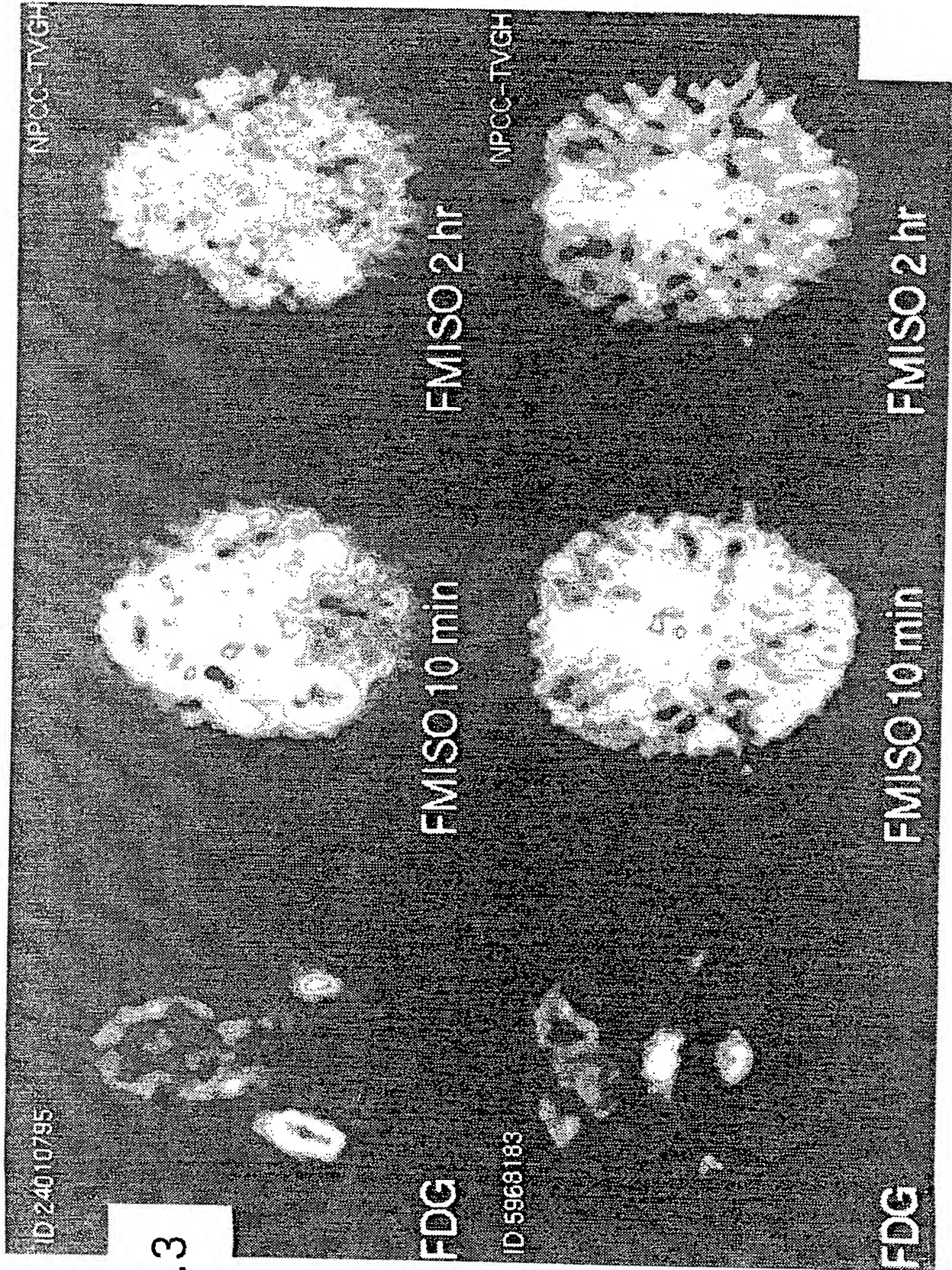


FIG. 3

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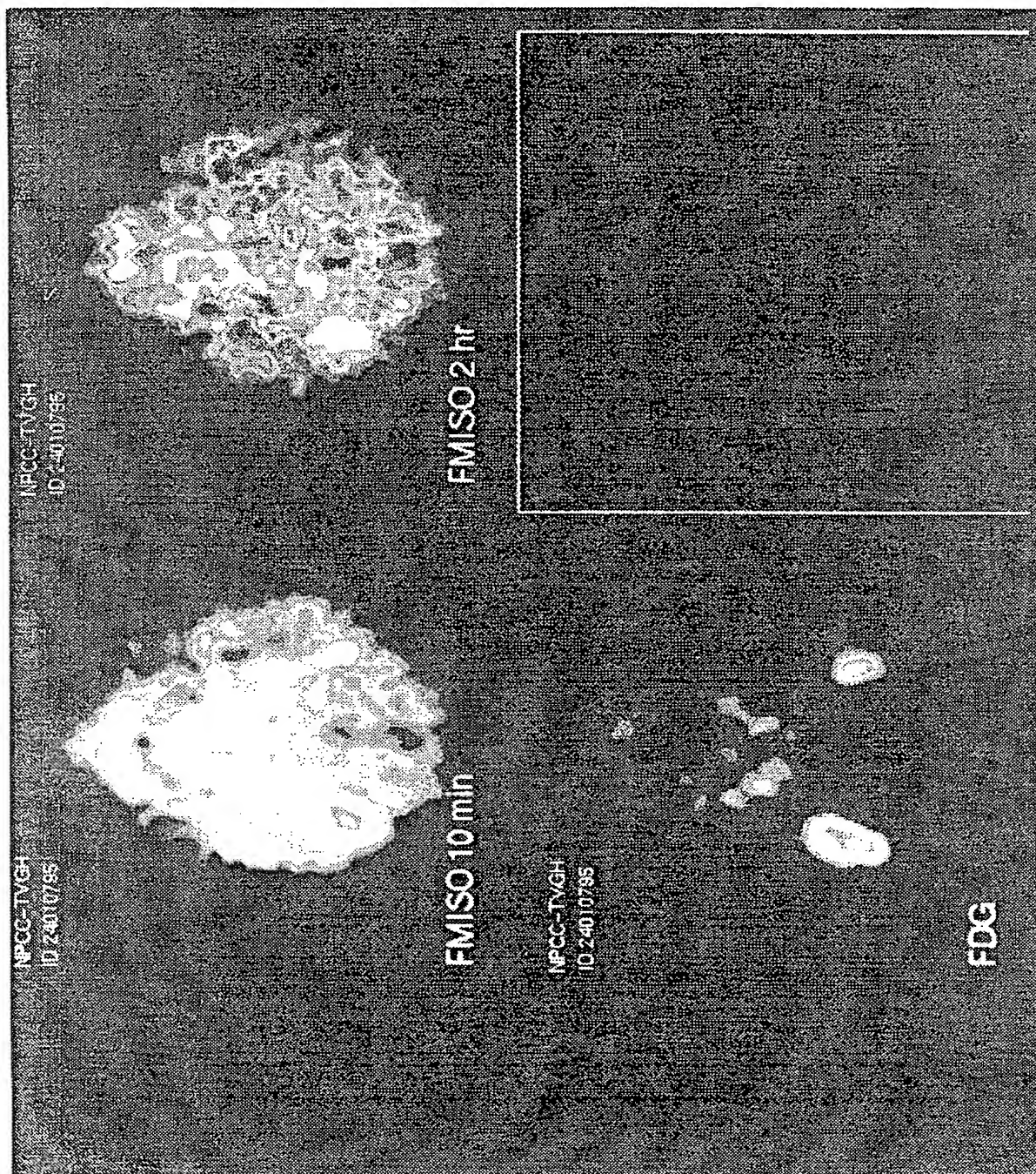


FIG.4

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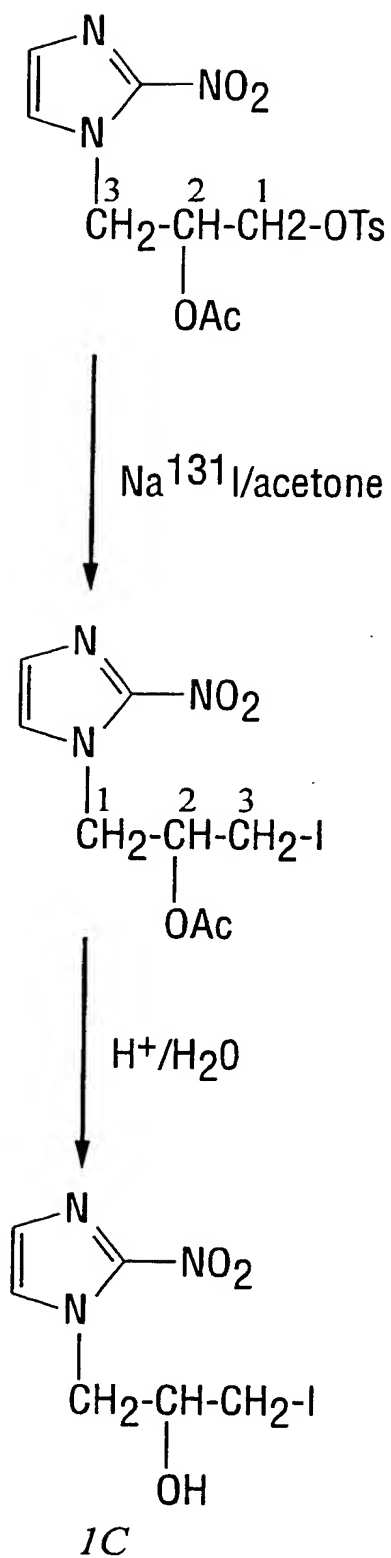
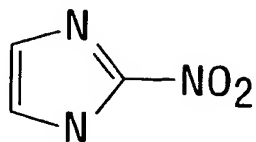


FIG. 5

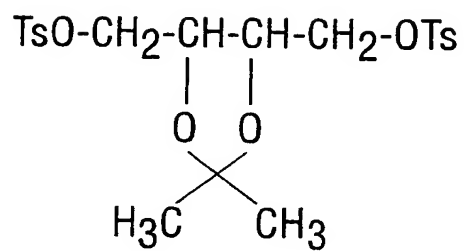
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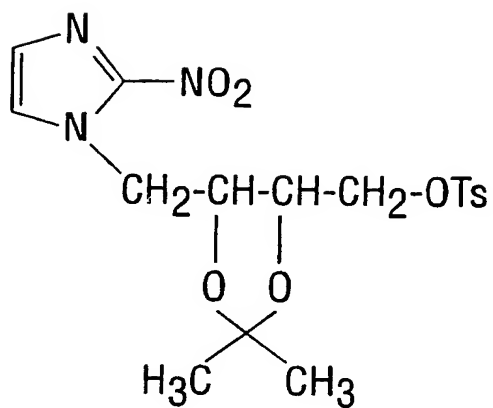
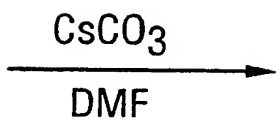


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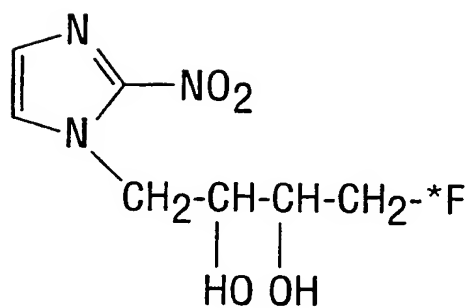
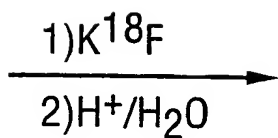
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[<sup>18</sup>F]FETNIM

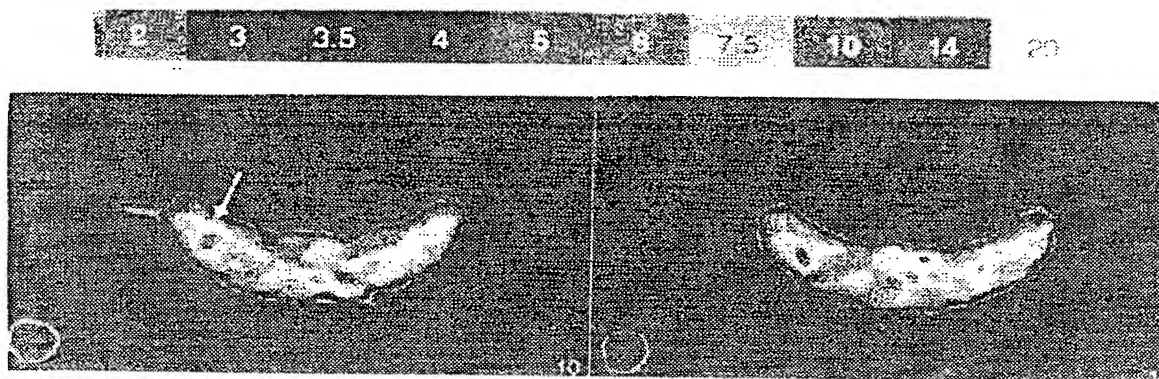
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FIG. 6

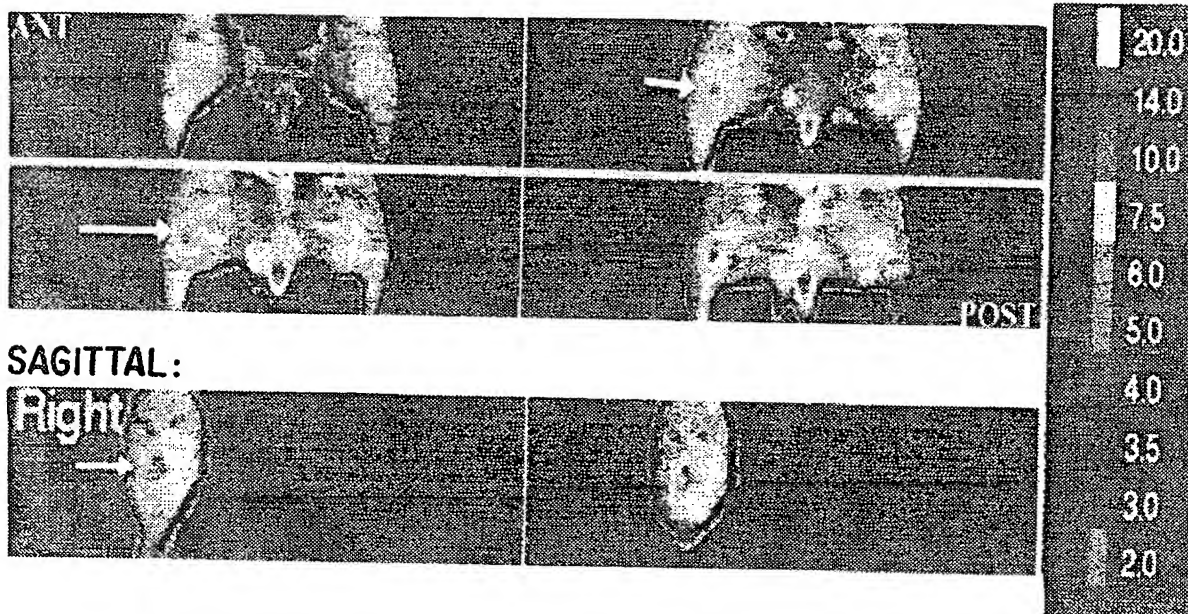


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TRANSAXIAL:



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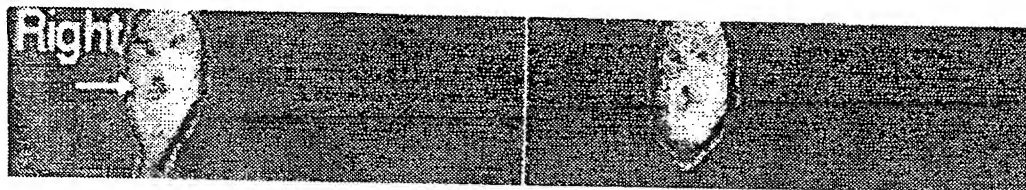


FIG. 7

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 $[^{18}\text{F}]$ FETNIM $[^{18}\text{F}]$ FDG $[^{18}\text{F}]$ FMISO

FIG. 8

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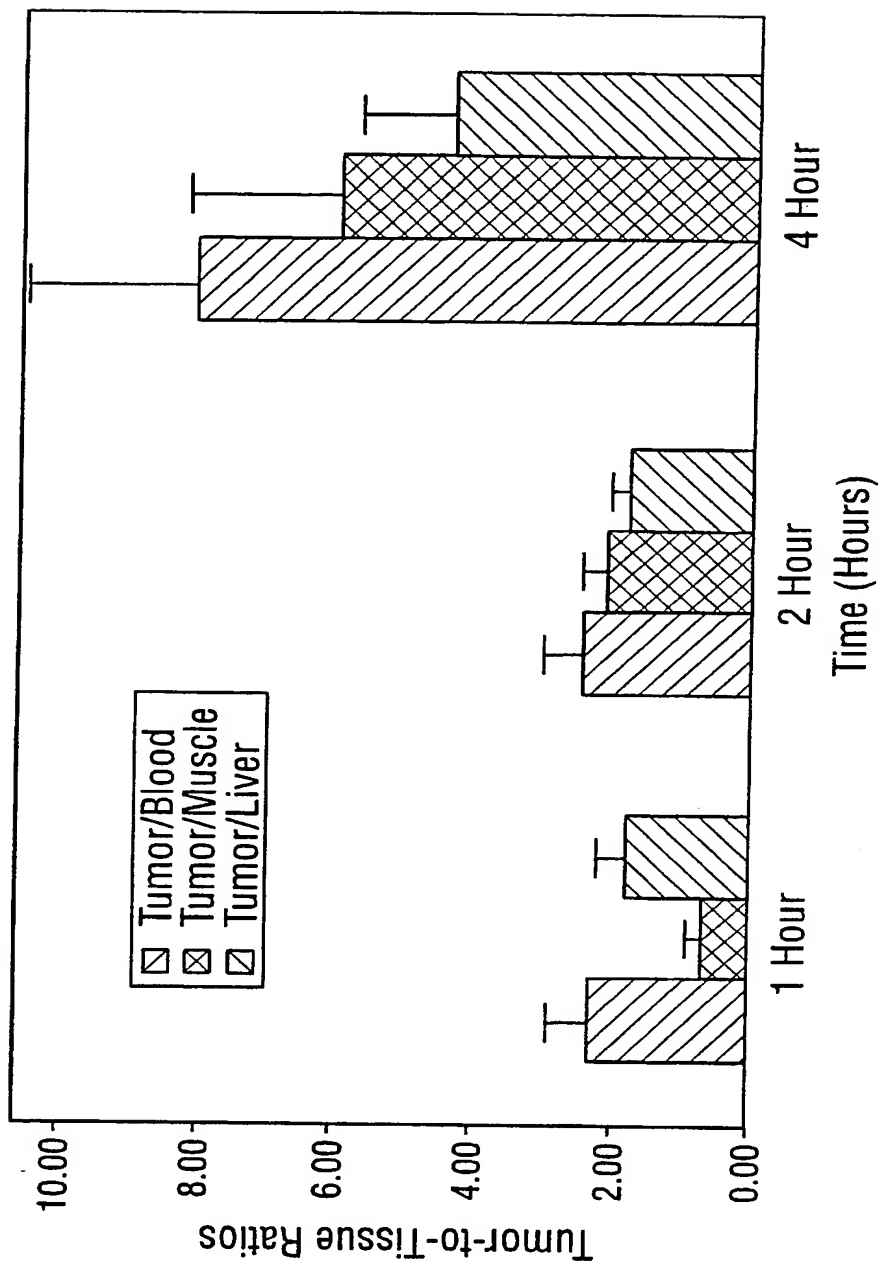


FIG. 9

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 94/11246

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C07D233/91 C07D405/06 C07B59/00 A61K51/04

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D C07B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	APPLIED RADIATION AND ISOTOPES, vol.44, no.8, August 1993, OXFORD GB pages 1085 - 1091 J.-L. LIM ET AL. 'An Efficient Radiosynthesis of [18F]Fluoromisonidazole' see the whole document ---	1-23
A	APPLIED RADIATION AND ISOTOPES, vol.40, no.2, 1989, OXFORD GB pages 117 - 126 D.-R. HWANG ET AL. 'No-carrier-added Synthesis of 3-[18f]Fluoro-1-(2-nitro-1-im idazolyl)-2-propanol. A Potential PET Agent for Detecting Hypoxic but Viable Tissues.' cited in the application see the whole document --- -/--	1-23

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

10 February 1995

Date of mailing of the international search report

22. 02. 95

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Fink, D

## INTERNATIONAL SEARCH REPORT

Int: International Application No

PC: 94/11246

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>CHEMICAL ABSTRACTS, vol. 121, no. 1, 4 July 1994, Columbus, Ohio, US; abstract no. 9252h, A. CHERIF ET AL. 'Rapid synthesis of 3-[18 F]fluoro-1-(2'-nitro-1'-imidazolyl)-2-prop anol ([18F]fluoromisonidazole).' page 982 ;column 2 ; see abstract &amp; PHARM. RES., vol.11, no.3, 1994 pages 466 - 469</p> <p>-----</p>	1,2,5,9

